REMARKS

Applicants submit this response to the Office Action dated January 13, 2003 (Paper No. 9). Claims 1, 6, 7, 8, 9, 10, 12, 19 and 20 are pending. Applicants thank the Examiner for indicating that claims 12, 17, 19 and 20 are included with Group IV, which was elected in Paper No. 8, filed October 28, 2002. Claims 1 and 17 are canceled in view of the election of Group IV (NET-4 inhibitory antibodies). Claims 6, 9, 12 and 20 are amended to reflect this election and no new matter is added.

Claims 1, 6-10, 12, 17, 19 and 20 are rejected under 35 U.S.C. § 112, first paragraph, as the specification allegedly is not enabling for making or using therapeutically effective amounts of NET-4 antibody modulators. The Examiner has indicated that one of skill in the art is enabled to make an antibody to NET-4. The issue therefore is whether such antibodies function as modulators of NET-4. Without acquiescing to the ground of rejection, applicants submit that the claims as amended (cancellation of claim 1, and amendment of claims 6, 9, 12 and 20) to reflect that the modulator is an antibody or a fragment thereof, are not subject to this ground of rejection to the extent that it pertains to "modulator" language. The Examiner's statements regarding the *enablement* of antibody modulators are addressed below.

1. **Tetraspan protein expression correlates with the cancer phenotype**

The Examiner has questioned the enablement of an antibody that inhibits the function of NET-4. To address this, applicants rely on the disclosure in the present specification, and the relevant scientific literature. Applicants submit that the data in the patent application and the information disclosed in the scientific literature together strongly support a conclusion that NET-4 expression plays a role in the phenotype of cancer cells, particularly cell growth.

Applicants submit that the data in the specification clearly show that inhibition of NET-4 with antisense oligonucleotides specific for NET-4 inhibits the growth of colon cancer cells. See, in particular, Example 2 at pages 33-35, and Example 4 at pages 39-40. The specification further discloses that the expression level of NET-4 in colon tumor cells is at least 2-fold greater than that of matched normal colon cells, as described in Example 5 at pages 40-43. Furthermore, the results of *in situ* hybridization studies of NET-4 expression in normal colon and lung tissues, as compared with colon tumor and lung tumor tissues, indicate increased expression in the tumor tissues (see Example 6 at page 43, and Figure 2). Therefore, the application has provided a detailed study of NET-4 expression in tumor cells, and this expression is consistent

with applicants' discovery that inhibition of NET-4 expression using antisense oligonucleotides specific for NET-4 has an inhibitory biological effect on tumor cells.

The antisense oligonucleotides that were employed in the experiments disclosed in the patent application are specific to the coding region of NET-4 polynucleotide, as shown in SEQ ID NO:1 of the application. Thus, it can be reasonably asserted that the inhibition results in a decrease in NET-4 protein production. NET-4 is a member of the family of tetraspan proteins, as described in the specification at page 1, lines 12-15, and page 3, lines 2-21, and references cited therein. Tetraspan protein expression has been correlated with the cancer phenotype in publications that pre-date the filing date of the present application. For example, Tachibana *et al.* reported that members of the tetraspan membrane 4 superfamily are expressed in cells of a human breast cancer cell line, as demonstrated using monoclonal antibodies. The protein recognized by the antibodies was identified as NAG2 protein, which co-localized with CD81 on the cell surface of breast cancer cells. (Tachibana, I. *et al.*, *J. Biol Chem.* 272:29181-29189, 1997.)

More recently, Burchert *et al.* reported that a member of the tetraspan family, CD82, was overexpressed in cells of patients with chronic myeloid leukemia, acute myeloid leukemia, and chronic lymphocytic leukemia. Expression levels were reduced when cells were induced to differentiate. (Burchert, A. *et al.*, *Br. J. Haematol.* 107:494-504, 1999.) Thus, the correlation that applicants have observed between increased NET-4 expression and a cancer phenotype is consistent with published reports on other tetraspan proteins. Burchert's report that differentiation of leukemia cells correlated with a decrease in CD82 protein expression is consistent with applicants' discovery that inhibition of NET-4 expression reduced tumor cell proliferation.

Furthermore, tetraspan proteins have been the target of anti-tumor therapeutic antibodies. For example, Oren *et al.* found that antibodies to a protein referred to as TAPA-1 (CD81) inhibited the growth of tumor cell lines *in vitro*. (Oren, R. *et al.*, *Mol. Cell Biol.* 10:4007-4015, 1990.) Mice were immunized with lymphoma cells from patients, or with cell lines derived from patients. Hybridomas were prepared using immune cells from these mice. Antibodies from the hybridomas were tested for their ability to inhibit the proliferation of a human lymphoma cell line. Further analysis led to the identification of a tetraspan protein (TAPA-1) that was recognized by several hybridomas. These hybridomas had an anti-proliferative effect on several lymphoma cell lines, all of which were from B-cell lymphomas. The TAPA-1 tetraspan protein was

identified by cloning, sequencing of the DNA, and comparison of the open reading frame with that of other tetraspan protein family members. Studies like those of Oren *et al.* validate tetraspan proteins as targets for antibodies that inhibit cell proliferation. Applicants' data support NET-4 as another such target.

2. **Inhibition using antisense correlates with inhibition of the same protein using antibodies**

The Examiner stated that it was unclear that a correlation could be drawn between inhibition using antisense oligonucleotides, and inhibition using antibodies, because antisense targets nucleic acids, and antibodies target proteins. (Office Action at page 4, last paragraph, lines 4-6.) It is applicants' position that the data in the specification, coupled with the knowledge in the art regarding the correlation between tetraspan expression and tumor phenotype and growth, enables one of skill in the art to produce antibodies that would be expected to have a therapeutically inhibitory effect on tumor cells.

As evidenced by the accompanying Declaration of Dr. A. B. Jefferson under 37 C.F.R. § 1.132, one of skill in the art would expect such correlation. For example, Dr. Jefferson cites a publication showing that inhibition of CD44 expression in tumor cells was accomplished using antibodies and antisense oligonucleotides, and the targeting of CD44 resulted in reduction of the malignant activities of the tumor cells. (Naor *et al., Crit Rev. Clin Lab.*, 39:527-579, 2002.) Naor describes studies in which treatment of animals with anti-CD44 antibodies suppressed a variety of malignant activities. At page 555, Naor states, "[d]ownregulation of tumor-supporting CD44 by specific antisense transfection *is an alternative way of proving that CD44 (especially CD44v) targeting is a rational approach to cancer therapy.*" (Emphasis added.) Although there were some results specific to the biological role of CD44 in particular, the overall effect was a reduction in tumor spread.

Dr. Jefferson also discusses a publication by Pomerantz *et al.*, who reported that blocking of epidermal growth factor receptor (EGFR) with monoclonal antibodies, and with antisense oligonucleotides, is being investigated for anti-cancer therapy, in view of the upregulation of EGFR in many types of human tumors. (Pomerantz *et al.*, *Curr. Oncol. Rep.* 5:140-146, 2003.) Pomerantz reported studies showing that antisense oligodeoxynucleotides targeting the translation start sites of EGFR inhibited the proliferation of head and neck squamous

carcinoma cells (HNSCC). In other studies, antisense constructs directed against EGFR inhibited tumor growth when administered intratumerally to HNSCC xenografts in nude mide.

Pomerantz *et al.* also reported that monoclonal antibodies specific for EGFR inhibited the growth of HNSCC cell lines, and these studies led to human studies of anti-EGFR antibody therapy of HNSCC.

Dr. Jefferson also discusses another protein family that is important in normal and tumor cell growth, vascular endothelial growth factor, or VEGF. This protein family includes VEGF-C, which is implicated in malignant mesothelioma growth. According to Dr. Jefferson, Masood *et al.* found that antisense oligonucleotide complementary to VEGF inhibited VEGF expression and also specifically inhibited mesothelioma cell growth. Antibodies to VEGF receptor also inhibited mesothelioma cell growth. Although in this case the antibodies and antisense were directed to different proteins, the two proteins are functionally related (protein and its receptor), indicating that the ultimate effect was to prevent the protein from carrying out its normal biological role. (Masood *et al., Int. J. Cancer* 104:603-610, 2003.)

Finally, Dr. Jefferson discusses a report by Stearns *et al.*, that inhibition of insulin-like growth factor (IGF) receptor using antisense specific for IGF receptor polynucleotides, and using IGF receptor-specific antibodies, had similar effects on the ability of IL-10 to block IGF activation of mRNA expression and protein synthesis in cancer cells. (Stearns *et al., Clin. Cancer Res.* 9:1191-1199, 2003.)

Thus, as attested to by Dr. Jefferson, there is a strong, art-recognized correlation between inhibition of protein expression using specific antisense oligonucleotides, and inhibition of the same protein using specific antibodies. Thus, one of ordinary skill in the art would not doubt that a correlation can be drawn between applicants' demonstration of anti-tumor activity of NET-4 antisense oligonucleotides, and the use of anti-NET-4 antibodies as therapeutic antibodies as claimed.

For these reasons, reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph, are respectfully requested. The publications cited in the response and declaration are made of record on the accompanying form PTO/SB/08.

Applicants submit that the application is in condition for allowance and respectfully request issuance of a Notice of Allowance.

If questions remain regarding this application, the Examiner is invited to contat the undersigned at (206) 628-7650.

Respectfully submitted,
Christoph Reinhard et al.
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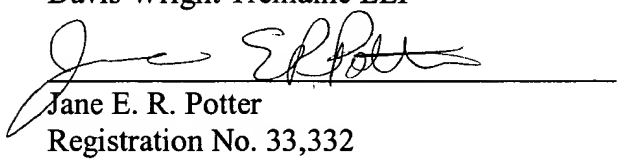

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